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Telomere Shortening in Hematopoietic Stem Cell Transplantation: A Potential Mechanism for Late Graft Failure?

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ABSTRACT

Telomeres serve to maintain the structural integrity of chromosomes, yet each somatic cell division is associated with a decrease in telomere length. The cumulative decrease in telomere length can impose an upper limit for the number of cell divisions that can occur before a cell senesces. When studied in vitro with fibroblasts, this limit is referred to as the Hayflick limit and usually occurs after 40 to 80 cell doublings. In theory, a similar replicative potential in a hematopoietic stem cell could support hematopoiesis in a person for more than 100 years. However, stem cells differentiate, and the telomere length differs among chromosomes within a single cell, among cell types, and among age-matched individuals. This variation in telomere length raises the possibility that long-term hematopoiesis by transplanted stem cells could, depending on the telomere length of the engrafted stem cell and the proliferative demand to which it is subjected, reach a Hayflick limit during the life span of the patient. Although significant shortening of telomeres is reported to occur within the first year posttransplantation, as yet no evidence has indicated that this shortening is associated with marrow function. In this review, we summarize reports on telomere shortening in stem cell transplantation recipients and report 2 cases in which graft failure is associated with significant telomere shortening.

KEY WORDS

Telomerase • Stem cells • Transplantation • Telomere length

INTRODUCTION

What Are Telomeres?

Telomeres are the noncoding regions of DNA that provide a protective cap at the ends of eukaryotic chromosomes. Telomeres protect encoding DNA from enzymatic breakdown and prevent dicentric fusion and other chromosome aberrations. The mechanism for regulating telomere length is not entirely known but is partly attributed to the ribonucleoprotein reverse transcriptase, known as telomerase [1]. It has been shown that telomeres shorten 50 to 150 base pairs (bp) per cell division in most somatic tissues, which, in general, do not express telomerase [2-4]. In contrast, telomeres do not progressively shorten in germ line tissues that do express telomerase [3]. Induction of telomerase appears to maintain telomere length and postpone senescence [5-7]. Constitutive expression of telomerase is generally a requirement for immortalization of cells [8-10].

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TELOMERE SHORTENING AFTER HEMATOPOIETIC STEM CELL TRANSPLANTATION

Several investigators have addressed the issue of telomere shortening in nucleated blood cells of stem cell transplant recipients. It was hypothesized that replicative demand imposed on donor stem cells may result in substantial shortening of telomeres, thereby potentially compromising the long-term function of the marrow graft. Most studies involving adult and pediatric patients have

Table 1. *Telomere Length Measurements after Transplantation*

	Age	Cell Type	Median Telomere Length, kb	Normal Telomere Length at Age (50th Percentile), kb*
Donor	64	Monocytes	9.9†	10.1
		Lymphocytes	8.3†	7.8
		Naïve T-cells	8.3†	8.3
		Memory T-cells	7.2†	7.1
		B-cells	10.3†	9.8
Recipient	10	Monocytes	8.3‡	12.5
		Lymphocytes	6.5‡	11.7
		Naïve T-cells	6.4‡	12.1
		Memory T-cells	6.4‡	10.7
		B-cells	8.3‡	12.0

*Values are based on the data from 400 healthy individuals aged 0 to 102 years (G.M.B and P.M.L., unpublished data).

†Normal telomere length: values between the 10th and 90th percentile of age-matched controls.

‡ Very low telomere length: values below the first percentile of age-matched controls.

determined that the telomere length of cells in the marrow graft was 0.4 to 2 kb shorter than the pretransplantation telomere length detected in the donor, although the source of samples (ie, sorted or unsorted leukocytes, neutrophils, or mononuclear cells from peripheral blood) and the observation period varied among studies [11-14]. Mathioudakis et al. looked at 17 marrow transplantation survivors with a follow-up of 19 to 28 years and found telomeres shortened on average 0.94 kb [15]. More recent data obtained from 25 stem cell transplant recipients showed that, on average, a 0.5-kb decrease in telomere length occurred within the first year [16] with very little, if any, decrease thereafter [17]. This observation supports the notion that an increased replicative demand on stem cells during the period of hematopoietic reconstitution results in measurable telomere shortening. This finding is also supported by data indicating that the extent of telomere shortening is inversely correlated with the number of nucleated cells transplanted [11,14].

Although most studies indicate some degree of accelerated telomere shortening in transplant recipients, the degree of shortening, at least for the majority of patients, does not appear to reach a level that compromises marrow function. This circumstance could change with longer follow-up or in cases in which very few donor stem cells were transplanted or in which donor cells, because of age or unknown reasons, have unusually short telomeres. In these instances, it is reasonable to speculate that telomere shortening could reach a critical level resulting in senescence of hematopoietic cells that, in turn, could compromise marrow function. A linkage between telomere dysfunction and marrow failure is strongly supported by recent data indicating that haploinsufficiency for the telomerase RNA gene typically results in aplastic anemia [18]. In this review, we summarize 2 cases in which telomere shortening may have played a role in poor graft function.

CASE 1: OLD MARROW INTO A YOUNG RECIPIENT

A 7-year-old male patient received a bone marrow transplant from his paternal grandmother for acute lymphoblastic leukemia (ALL) in second complete remission (CR). The age of the donor was 61 years. Pretransplantation conditioning consisted of 1440 cGy total body irradiation (TBI) and cyclophosphamide (120 mg/kg). A total of 4.04×10^8/kg nucleated cells was infused. Donor and recipients were serologically identical at HLA-A, -B, and -C, with a single-allele, unidirectional mismatch at DRB1 in the direction of graft-versus-host disease (GVHD). The patient's peripheral blood counts showed stable engraftment. Cytogenetic and fluorescence in situ hybridization (FISH) analyses of the bone marrow revealed all donor-derived cells during the entire course of treatment. Despite successful engraftment of the donor cells, the patient experienced low absolute neutrophil counts (800-900/mm^3) at 25 months after transplantation, with a further drop to 302/mm^3 at 32 months. The hematocrit and platelet levels also decreased to 25% and 125,000/mm^3, respectively. At the time of this report, the patient needed granulocyte colony-stimulating factor and erythropoietin to maintain his neutrophil and red cell counts. Poor marrow function could not be attributed to the most common causes of myelosuppression, including drugs, viral infections, relapse, rejection, or GVHD.

Considering the donor age at the time of transplantation, we measured telomere length in subsets of leukocytes obtained from peripheral blood of the recipient and donor (Table 1) using FISH and flow cytometry [19,20] with modifications that allow analysis of subsets within the same sample ([21] and G.M.B. and P.M.L., unpublished data). Telomere length values in subsets of cells obtained from the donor were comparable to those of age-matched control values for the same cell populations derived from a reference group of 400 healthy individuals. However, posttransplantation donor cells harvested from the patient were 0.8 to 2.0 kb shorter. This finding indicates that this young patient has a hematopoietic system with telomere lengths that are significantly shorter than those of his age-matched controls.

CASE 2: A LONG-TERM SURVIVOR WHO DEVELOPED MARROW FAILURE

This patient underwent an HLA-matched transplantation of bone marrow from his sister for the treatment of severe aplastic anemia. At the time of transplantation the patient was 13 years old and the donor was 14 years old. The patient received 200 mg/kg of cyclophosphamide as a preparatory regimen and methotrexate for GVHD prophylaxis for 3 months. A total of 2.9×10^8/kg nucleated cells was infused. The transplant engrafted well and the patient had normal blood counts until 25 years after transplantation, when abnormal counts were noted: white blood cells 1100/mm^3, absolute neutrophil counts 310/mm^3, hematocrit 33.3%, and platelet counts 32,000/mm^3. The patient's bone marrow was shown to be hypocellular. Although hepatitis C also had been diagnosed and the patient showed signs of hypersplenism, the results of bone marrow examination excluded the possibility that hypersplenism caused the pancytopenia. FISH analysis results of marrow and peripheral

Table 2. *Telomere Length Measurements after Transplantation*

	Age	Cell Type	Median Telomere Length, kb	Normal Telomere Length at Age (50th Percentile), kb*
Donor	**42**	**Mononuclear cells**	**7.4†**	**8.7**
Recipient	**40**	**Mononuclear cells**	**5.2‡**	**8.8**

*Values are based on the data from 400 healthy individuals aged 0 to 102 years (G.M.B. and P.M.L., unpublished data).

†Normal telomere length: values between the 10th and 90th percentile of age-matched controls.

‡Very low telomere length: values below the first percentile of age-matched controls.

blood showed more than 96% donor-derived hematopoiesis. When the patient developed pancytopenia, telomere length in blood mononuclear cells of the recipient was an estimated 2.2 kb shorter compared to that of the donor (recipient, 5.2 kb; donor 7.4 kb) (Table 2).

TELOMERE SHORTENING: A POSSIBLE MECHANISM FOR LATE GRAFT FAILURE?

Several cross-sectional studies have shown that the telomere length in nucleated blood cells from healthy individuals shortens with age at a rate of 30 to 50 bp per year [12-14,22,23]. It is tempting to use this type of data to translate the degree of telomere loss observed in transplant recipients into equivalent years of normal aging. In the 2 cases that were presented here, this type of calculation yielded 27 to 67 years of "telomere aging" in case 1 and 44 to 73 years in case 2. However, there are several pitfalls in this type of calculation. First, the figure for the telomere loss with age is calculated using cross-sectional rather than longitudinal data. Given the marked variation in telomere length at any given age, the rates of shortening could differ between healthy individuals. Longitudinal data are essential to address this issue but are currently not available. Furthermore, it is known that the loss of telomeres in leukocytes with age is not linear and, for example, occurs at a much higher rate in children than in adults [20,24]. Although such differences have been primarily related to stem cell turnover [20], it is becoming increasingly clear that oxidative stress is an important contributor to telomere loss (reviewed in [25]). Such nonreplicative telomere shortening complicates the use of telomere length in cells as a measure of their mitotic or chronological age. Finally, the telomere length at which cells are unable to continue cell division may vary between individuals [26], depending on, for example, the expression level of telomere binding proteins [26] and/or levels of functional telomerase. The latter is illustrated in tumor cells and immortalized cell lines, which can have short telomeres that are stabilized by telomerase [8].

On the other hand, several studies support the idea that telomere shortening in hematopoietic stem cells may compromise their function and the ability of the hematopoietic system to meet proliferative demands. Patients with the rare disorder dyskeratosis congenita typically die before the age of 50 from complications of aplastic anemia, immune deficiency, or cancer (reviewed in [27]). Patients with this disorder typically show decreased telomerase activity, eg, resulting from a mutation in the telomerase RNA component (TERC) gene [18]. Strikingly, such a defect gives rise to an autosomal dominant disorder, indicating that even partial telomerase deficiency is poorly tolerated in humans. In contrast, complete lack of telomerase is tolerated for up to 6 generations in mice [28]. Most likely, telomere shortening evolved as a tumor suppressor mechanism in long-lived species (reviewed in [29]). Telomere length data in patients with dyskeratosis congenita and aplastic anemia (see, eg, [30-33]) provide strong support for a linkage between replicative potential and telomere length in hematopoietic stem cells.

Problems related to telomeres in recipients of stem cell transplants are to some extent predictable and in most cases preventable. Transplantation of a very limited number of stem cells will put high demands on their proliferative potential. This situation may eventually result in exhaustion of stem cells and aplastic anemia, especially if telomeres in donor stem cells were short to start with. In this respect cord blood cells may have a significant advantage that may outweigh concerns about the limited number of cells that are typically available for transplantation. Long-term survivors of stem cell grafts containing limited numbers of stem cells (or large numbers of compromised stem cells) could be particularly at risk if extra demands are put on the hematopoietic system, eg, as a result of chronic infection.

Currently no precise associations have been discovered between telomere lengths on a specific chromosome and a specific functional abnormality. Therefore exactly what constitutes functionally relevant telomere shortening in the transplantation setting remains to be determined. Regarding the example provided by "Old Marrow into Young Recipient," one might argue that aplasia due to telomere shortening should not be responsive to growth factors, but we do not know enough to make that assumption. Growth factors might provide a survival signal to an otherwise compromised cell. It is reasonable to speculate in this case, however, that treatment with growth factors will only accelerate telomere shortening, leading eventually to greater genetic instability, loss of responsiveness to growth factors, and/or apoptosis. More data are urgently needed on telomere length associated with various subpopulations of hematopoietic cells in healthy individuals and patients with hematological disorders and with age and disease progression. Given the increasing number of long-term survivors of stem cell transplantation and the increasing age of donors, it is important to consider that loss of telomeres in donor stem cells could eventually compromise marrow function.

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